GENETIC FINGERPRINTING OF VARIOUS NATIVE CALIFORNIA CULTIVARS

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ABSTRACT

We employed isozyme and random amplified polymorphic DNA (RAPD) markers to determine the genotypes or "genetic fingerprints" for five groups of cultivars, each of which contain two or more varieties suspected of being identical. Cultivars previously hypothesized to be identical were found to differ isozymically in only one case (*Ceanothus* 'Dark Star' and 'Julia Phelps'). In contrast, only the cultivar pairs *Salvia mellifera* 'Prostrata' and 'Tera Seca' and *Fremontodendron* 'Ken Taylor' (Tree of Life) and 'El Dorado Gold' displayed identical RAPD profiles. Based on these results, we place *Salvia mellifera* 'Prostrata' in synonymy with *Salvia mellifera* 'Tera Seca' because the latter represents the first nomenclaturally valid name for this cultivar. Likewise, *Fremontodendron* 'Ken Taylor' (Tree of Life) has been incorrectly identified and should be treated as synonymous with *Fremontodendron* 'El Dorado Gold'.

Native California cultivars constitute a major portion of the horticultural trade specializing in the propagation and distribution of native plants. Unlike native plants, however, cultivars are produced artificially, being derived from clones, crosses or self-fertilized pure lines. Consequently, the production of new varieties by artificial means requires a system of nomenclature suitable for distinguishing cultivated plants from their wild (native) counterparts. While the rules governing the nomenclature of wild and cultivated plants are basically similar, cultivated plants are treated under separate guidelines set forth in the *International Code of Nomenclature of Cultivated Plants.*

Despite a formal system for naming cultivars, no reliable means of ensuring the stability of cultivar names exists. For example, cultivars are often renamed by various nurseries trading in cultivated plants. Distribution to the public further complicates the problem as cultivated varieties can be transported out of state and reintroduced under a different name. Complications such as these lead to misidentification of cultivars, and confusion regarding their origins.

The purpose of this study is to investigate the identity of five groups of cultivars each of which contains two or more varieties

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suspected of being identical. In each case the hypothesis that two or more cultivars are derived from a single clone is based on morphological and phenological observations coupled with insufficient information regarding their origins or the possibility that due to their similarity they have been confused over time in the horticultural trade. The putatively identical varieties include *Heuchera* 'Susanna' and *H.* 'Santa Ana Cardinal' (group 1), *Salvia mellifera* E. Greene 'Little Sur', *S. mellifera* 'Prostrata', and *S. mellifera* 'Tera Seca' (group 2), *Ceanothus* 'Dark Star' and *C.* 'Julia Phelps' (group 3), *Ceanothus* 'Skylark' and *C.* 'Victoria' (group 4) and *Fremontodendron* 'Ken Taylor' (Tree of Life) and *F.* 'El Dorado Gold' (group 5). We also assayed one or more related but genetically distinct individuals for each group to ensure that the techniques we employed provided adequate resolution (hereafter referred to as control genotypes).

In order to distinguish the various cultivars, we employed isozyme and random amplified polymorphic DNA (RAPD) markers (Williams et al. 1990) to determine genotypes for the putatively identical cultivars. Determination of the genotype or "genetic fingerprint" of each cultivar will allow identification of clones. That is, if two or more cultivars exhibit identical genotypes, then the morphology-based hypothesis that they are identical is supported. Alternatively, cultivars which are found to be genotypically dissimilar refute this hypothesis and provide evidence in favor of maintaining their unique cultivar names.

Methods

Collections. Fresh leaf tissue was collected at Rancho Santa Ana Botanic Garden, Claremont, CA of *Heuchera maxima* E. Greene, *H.* 'Susanna', *H.* 'Santa Ana Cardinal', *Salvia mellifera* 'Point Mugu', *S. mellifera* 'Little Sur', *S. mellifera* 'Prostrata', *S. mellifera* 'Tera Seca,' *Ceanothus impressus* Trel. 'Vandenberg', *C.* 'Dark Star', *C.* 'Julia Phelps', *C. thyrsiflorus* Eschsch. 'Snow Flurry', *C.* 'Skylark', *C.* 'Victoria', *Fremontodendron californicum* (Torrey) Cov. ssp. *decumbens* (R. Lloyd) Munz 'Claremont', *F.* 'Ken Taylor' (Tree of Life), *F.* 'Ken Taylor' (UC Santa Cruz), *F.* 'West Hills Hybrid', *F.* 'El Dorado Gold'.

Enzyme electrophoresis. The following 12 enzymes were analyzed: acid phosphatase (ACPH), aspartate aminotransferase (AAT), glucose-6-phosphate isomerase (GPI), glutamate dehydrogenase (GDH), isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), malic enzyme (ME), phosphoglucomutase (PGM), phosphogluconate dehydrogenase (PGD), ribulose-bisphosphate carboxylase (RBC), shikimate dehydrogenase (SKD), and triose-phosphate isomerase (TPI).

Electrophoretic procedures generally followed those of Soltis et al. (1983). Leaf tissue was prepared using the Tris-HCl grinding buffer-PVP solution of Soltis et al. (1983). Enzymes were best resolved using the following buffer systems: for TPI, RBC, and AAT, system 6 (Soltis et al. 1983); GPI, GDH, and ME, system 8 (Rieseberg and Soltis 1987); PGD, PGM, and SKD, system 9 (Soltis et al. 1983); and ACPH, MDH, and IDH, system 1 (Soltis et al. 1983).

Genetic interpretation of isozyme phenotypes was based on knowledge of the generally conserved enzyme substructure, compartmentalization, and isozyme number in higher plants (Gottlieb 1981, 1982). Loci were designated sequentially within each group of cultivars, with the most anodally migrating isozyme designated *1*, the next *2*, and so on. Likewise, alleles were designated sequentially, with the most anodally migrating allele designated *a*.

RAPD analysis. For each cultivar, genomic DNA's were isolated from 30 mg of fresh leaf tissue following Rieseberg et al. (1992), which is a modification of the method of Doyle and Doyle (1987). After isolation, the DNA's were further purified with the ELU-QUICK™ DNA Purification Kit (Schleicher & Schuell) and then quantified on a fluorometer.

For analysis of RAPD variation, all 18 DNA's were surveyed for the presence of amplification polymorphisms using 23 arbitrary 10-mer oligonucleotide primers: UBC290 (University of British Columbia Biotechnology Center), A8, A13, A20, B4, B7, B8, B13, B17, B20, C2, C6, C8, C10, C13, C15, C20, D2, D3, D5, D8, D18, D20 (Operon Technologies, Inc.). Primer sequences are given in Fritsch et al. (1993). These primers were chosen for study because they are known to amplify robustly in other flowering plants (Fritsch et al. 1993). Amplification conditions and cycle parameters followed Williams et al. (1990) except that the concentration of *Taq* DNA polymerase was doubled. Amplification products were separated by electrophoresis on 1.5% Tris-borate agarose gels and detected by staining with ethidium bromide.

To eliminate inadvertent scoring of artifactual RAPD variation (Ellsworth et al. 1993; Muralidharan and Wakeland 1993), we varied template and primer concentration by 20–25% in both directions relative to the initial concentration for potentially informative primers (Fig. 1). Only polymorphisms observed under all six different reaction conditions were scored.

Results

Isozymes. Genotypes of all varieties in each group studied are listed for scoreable enzymes and loci in Table 1. Cultivars appearing identical in each group based on morphology are in bold type. Eight or more loci were scoreable for each group, and multiple alleles were

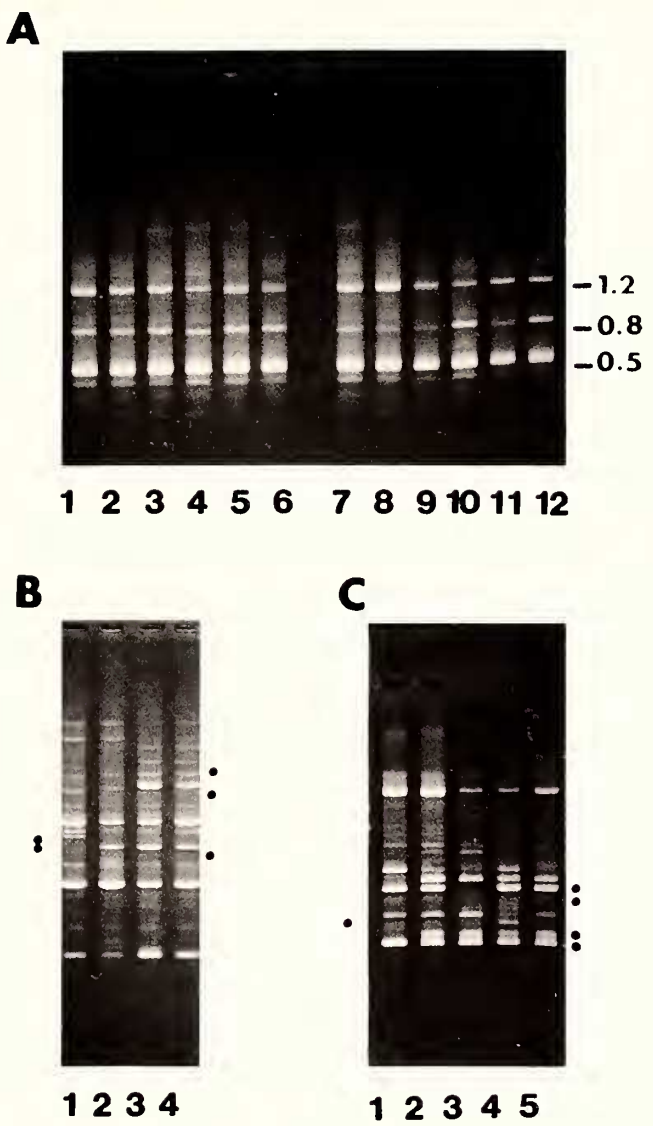

FIG. 1. Photographs of RAPD profiles in several native California cultivars. A) Amplification profiles for primer C13 in *Fremontodendron* 'Ken Taylor' (Tree of Life) (lanes 1–6) and *Fremontodendron* 'El Dorado Gold' (lanes 7–12) with varying concentrations of template and primer: lanes 1 and 7, 20 ng template; lanes 2 and 8, 25 ng template; lanes 3 and 9, 30 ng template; lanes 4 and 10, 0.15 μM primer; lanes 5 and 11, 0.2 μM primer; lanes 6 and 12, 0.25 μM primer. Fragment sizes in kb are given to the right of the photograph. Note relative constancy of RAPD profiles. B) Amplification profiles for primer A8 in the *Salvia* cultivar group: lane 1, *Salvia mellifera* 'Point Mugu'; lane 2, *S. mellifera* 'Little Sur'; lane 3, *S. mellifera* 'Prostrata'; and lane 4, *S. mellifera* 'Tera Seca'. Black dots adjacent to photograph indicate informative fragments. Sizes of informative fragments are in descending order: 2.1, 1.7, 0.95, 0.92, and 0.85 kb. C) Amplification profiles for primer B7 in the *Fremontodendron* group: lane 1, *F. californicum* ssp. *decumbens* 'Claremont'; lane 2, *F.* 'Ken Taylor' (Tree of Life); lane 3, *F.* 'Ken Taylor' (UC Santa Cruz); lane 4, *F.* 'West Hills Hybrid'; and lane 5, *F.* 'El Dorado Gold'. Black dots adjacent to photograph indicate informative fragments. Sizes of informative fragments are in descending order: 0.8, 0.75, 0.60, 0.55, and 0.50 kb.

TABLE 1. ISOZYME DATA FOR SELECTED CALIFORNIA CULTIVARS. Morphologically indistinguishable cultivars in each group are in bold. Control genotypes are marked with an asterisk.

Taxon	Cultivar	Locus/ genotype *Aat1*	*Aat2*
**Heuchera maxima*	#15,595	*aa*	*aa*
Heuchera	**'Susanna'**	*aa*	*aa*
Heuchera	**'Santa Ana Cardinal'**	*aa*	*aa*
**Salvia mellifera*	'Point Mugu'	*aa*	*aa*
S. mellifera	**'Little Sur'**	*aa*	*aa*
S. mellifera	**'Prostrata'**	*aa*	*aa*
S. mellifera	**'Tera Seca'**	*aa*	*aa*
**Ceanothus impressus*	'Vandenberg'	—	—
Ceanothus	**'Dark Star'**	—	—
Ceanothus	**'Julia Phelps'**	—	—
**Ceanothus thyrsiflorus*	'Snow Flurry'	—	—
Ceanothus	**'Skylark'**	—	—
Ceanothus	**'Victoria'**	—	—
**Fremontodendron californicum* ssp. *decumbens*	'Claremont'	—	—
Fremontodendron	**'Ken Taylor' (Tree of Life)**	—	—
**Fremontodendron*	'Ken Taylor' (UC Santa Cruz)	—	—
**Fremontodendron*	'West Hills Hybrid'	—	—
Fremontodendron	**'El Dorado Gold'**	—	—

found for at least two loci in each group. Cultivars previously hypothesized to be identical were found to differ isozymically in only one case (*Ceanothus* 'Dark Star' and 'Julia Phelps'). Although most cultivars differed by at least one polymorphism from the control genotype for their group, this was not the case for all members of the *Fremontodendron* group.

RAPD's. Much higher levels of polymorphism were observed for the RAPD amplification profiles. The control genotypes for each group differed from the cultivars by numerous reproducible markers. Likewise, several of the cultivars hypothesized to represent clones differed with respect to numerous RAPD polymorphisms (Table 2), including *Heuchera* 'Susana' and *H.* 'Santa Ana Cardinal' which differed by 22 polymorphisms, *Ceanothus* 'Dark Star' and *C.* 'Julia Phelps' which differed by 13 polymorphisms, and *Ceanothus* 'Skylark' and *C.* 'Victoria' which differed by 14 polymorphisms. In addition, *Salvia mellifera* 'Little Sur' differed from the other two *S. mellifera* cultivars by six polymorphisms (Table 2). In contrast, *Fre-*

TABLE 1. CONTINUED.

Locus/genotype												
Aat3	*Gpi1*	*Gpi2*	*Idh*	*Mdh1*	*Me*	*Pgd1*	*Pgd2*	*Pgm1*	*Pgm2*	*Rbc*	*Tpi1*	*Tpi2*
—	—	—	*aa*	*aa*	—	—	—	*bb*	*aa*	—	*aa*	*aa*
—	—	—	*aa*	*aa*	—	—	—	*aa*	*aa*	—	*ab*	*aa*
—	—	—	*aa*	*aa*	—	—	—	*aa*	*aa*	—	*ab*	*aa*
aa	*aa*	*ab*	—	*aa*	*aa*	—	—	—	—	*aa*	*ab*	*ab*
aa	*aa*	*bb*	—	*aa*	*aa*	—	—	—	—	*aa*	*ab*	*ab*
aa	*aa*	*bb*	—	*aa*	*aa*	—	—	—	—	*aa*	*ab*	*ab*
aa	*aa*	*bb*	—	*aa*	*aa*	—	—	—	—	*aa*	*ab*	*ab*
—	*aa*	*bb*	—	*aa*	—	*aa*	*aa*	*aa*	*aa*	*aa*	*aa*	*aa*
—	*aa*	*bb*	—	*aa*	—	*aa*	*aa*	*aa*	*ab*	*aa*	*ab*	*aa*
—	*aa*	*ab*	—	*aa*	—	*aa*	*aa*	*aa*	*ab*	*aa*	*ab*	*aa*
—	*aa*	*aa*	*aa*	*aa*	—	*ab*	*aa*	*aa*	*aa*	*aa*	*aa*	*aa*
—	*aa*	*aa*	*ab*	*aa*	—	*aa*	*aa*	*aa*	*aa*	*aa*	*aa*	*aa*
—	*aa*	*aa*	*ab*	*aa*	—	*aa*	*aa*	*aa*	*aa*	*aa*	*aa*	*aa*
—	*aa*	*aa*	*ab*	*aa*	—	—	—	*aa*	—	*aa*	*ac*	*aa*
—	*aa*	*aa*	*ab*	*aa*	—	—	—	*aa*	—	*aa*	*ac*	*aa*
—	*aa*	*aa*	*ab*	*aa*	—	—	—	*aa*	—	*aa*	*ab*	*aa*
—	*aa*	*aa*	*ab*	*aa*	—	—	—	*aa*	—	*aa*	*ab*	*aa*
—	*aa*	*aa*	*ab*	*aa*	—	—	—	*aa*	—	*aa*	*ac*	*aa*

montodendron 'Ken Taylor' (Tree of Life) and *F.* 'El Dorado Gold' were identical with respect to their RAPD profiles (Fig. 1) as were *Salvia mellifera* 'Prostrata' and 'Tera Seca' (Fig. 1; Table 2).

DISCUSSION

This study demonstrates the utility of isozymes and RAPD's for "genetic fingerprinting" plant cultivars suspected to be part of a single clone. Since many plants of horticultural importance are propagated asexually, all individuals of a cultivar are likely to belong to a single genet. All individuals in that genet will then be expected to yield identical isozyme and RAPD patterns. For example, the *Heuchera, Fremontodendron,* and *Ceanothus* cultivars analyzed herein are of hybrid origin and are by necessity propagated asexually primarily from cuttings (the *Heuchera* clones have been produced by tissue culture as well) to maintain their inherent unique characteristics. The *Salvia mellifera* clones are all prostrate forms which have been solely propagated from cuttings.

TABLE 2. RAPD DATA FOR SELECTED CALIFORNIA CULTIVARS. Morphologically indistinguishable cultivars in each group are in bold. Only data from reproducible and informative polymorphisms are given. Control genotypes are marked with an asterisk. The presence or absence of fragments is indicated by a "+" and "−", respectively.

		Primer:	UBC290			B17						C2				C8								C13						
Taxon	Cultivar	Fragment (kb):	1.5	1.1	0.8	1.5	0.90	0.85	0.70	0.40	1.4	0.70	0.65	0.55	0.51	1.35	1.3	1.0	0.9	0.8	0.7	0.5	0.4	1.5	1.1	0.76	0.68	0.66	0.6	0.45
*_Heuchera maxima_	#15,595		−	+	−	−	+	−	+	−	−	−	+	−	−	+	−	−	−	−	+	−	−	+	−	−	−	−	−	+
Heuchera	**'Susanna'**		+	−	+	−	−	−	+	+	+	+	−	+	−	−	−	−	−	+	−	+	−	−	+	−	−	−	+	−
Heuchera	**'Santa Ana Cardinal'**		−	+	−	+	+	+	−	+	−	−	−	−	+	−	+	+	+	−	−	−	+	−	−	+	−	+	−	+

		Primer:	A8					A20				B4		B8		C2			C8	C10			C13	C15	D2		D3	D8	D18	D20		
Taxon	Cultivar	Fragment (kb):	2.1	1.7	0.95	0.92	0.85	1.4	1.1	0.9	0.7	0.90	0.45	1.1	0.65	1.4	0.55	0.50	0.22	2.1	1.2	1.1	0.75	1.8	0.7	0.67	0.8	0.75	0.76	1.4	1.25	1.0
*_Salvia mellifera_	**'Point Mugu'**		−	−	+	+	−	−	+	+	+	−	+	−	−	+	−	+	−	+	−	−	−	+	+	−	−	−	+	−	+	−
S. mellifera	**'Little Sur'**		−	−	−	−	+	+	−	+	−	+	−	+	+	−	+	−	−	−	+	+	+	−	−	+	+	−	−	+	−	+
S. mellifera	**'Prostrata'**		+	+	−	−	+	+	−	−	−	+	−	+	−	−	+	−	+	−	+	+	+	−	−	+	+	+	−	+	−	+
S. mellifera	**'Tera Seca'**		+	+	−	−	+	+	−	−	−	+	−	+	−	−	+	−	+	−	+	+	+	−	−	+	+	+	−	+	−	+

		Primer:	A8				A20					B4					B7			D5	C2			C6	C13		D3	D18	
Taxon	Cultivar	Fragment (kb):	1.8	1.6	1.0	0.52	1.7	1.6	0.90	0.62	0.42	1.7	0.8	0.78	0.6	0.5	0.4	1.4	0.75	0.72	1.0	0.7	0.6	0.3	0.4	0.8	0.5	0.8	0.85	0.52
*_Ceanothus impressus_	**'Vandenberg'**		+	+	+	−	+	+	−	−	−	−	−	−	+	+	−	−	−	−	+	+	−	+	−	+	−	−	−	+
Ceanothus	**'Dark Star'**		−	−	−	+	−	−	+	+	+	+	−	+	−	−	−	+	−	+	+	−	−	−	−	−	+	+	+	+
Ceanothus	**'Julia Phelps'**		−	−	−	+	−	−	+	+	+	−	+	−	−	−	+	−	+	−	−	−	+	−	+	+	+	+	−	−

		Primer:	A13	A20			C6							B8			B17		C20			D3	D8		
Taxon	Cultivar	Fragment (kb):	0.52	1.1	0.85	0.52	0.9	0.85	0.8	0.7	0.65	0.5	0.4	1.3	1.2	0.90	0.80	0.6	0.55	1.4	0.8	0.6	0.46	1.0	0.65	0.45
*_Ceanothus thyrsiflorus_	**'Snow Flurry'**		−	−	+	+	−	+	−	+	+	+	−	+	−	−	+	+	−	+	−	+	−	+	+	−
Ceanothus	**'Skylark'**		+	+	+	−	+	+	+	−	−	+	+	−	−	+	−	−	+	−	+	+	+	−	−	+
Ceanothus	**'Victoria'**		+	+	−	−	−	−	−	+	−	−	−	−	+	+	−	−	+	+	−	−	−	+	−	−

		Primer:	A8		A20		C2		B7					B8			B17		B20	C8	D3
Taxon	Cultivar	Fragment (kb):	1.4	0.3	1.2	1.0	1.2	0.6	0.8	0.75	0.60	0.55	0.5	1.0	0.9	0.78	1.0	0.8	1.0	.78	0.7
*_Fremontodendron californicum_ ssp. *decumbens*	**'Claremont'**		−	+	+	−	+	−	−	+	+	−	−	−	−	−	+	−	−	−	−
Fremontodendron	**'Ken Taylor' (Tree of Life)**		−	+	−	+	−	+	+	+	+	−	+	−	−	+	−	−	−	+	−
*_Fremontodendron_	'Ken Taylor' (UC Santa Cruz)		+	−	−	−	−	+	+	−	+	−	+	−	+	−	+	−	+	+	+
*_Fremontodendron_	'West Hills Hybrid'		+	−	−	−	−	−	+	+	−	+	+	+	−	−	+	+	+	−	+
Fremontodendron	**'El Dorado Gold'**		−	+	−	+	−	+	+	+	+	−	+	−	−	+	−	−	−	+	−

To date, cultivar identification has been accomplished primarily with isozyme and morphological polymorphisms (e.g., Tanksley and Orton 1983; Granger 1993). However, these descriptors sometimes are not variable enough to distinguish individual genotypes. For example, *Heuchera* 'Susana' and *H.* 'Santa Ana Cardinal', *Ceanothus* 'Skylark' and *C.* 'Victoria', and *Salvia mellifera* 'Little Sur' and the other two *S. mellifera* cultivars were identical in terms of morphology and isozymes, but could be easily distinguished based on their RAPD amplification profiles. Nonetheless, these results should not be used to denigrate the utility of isozymes and morphology for cultivar identification and discrimination. Both types of data can be gathered more efficiently and with less expense than RAPD data. Furthermore, artifactual variation is much less of a problem for isozymes than RAPD's, although the opposite is true for morphological data. Moreover, if cultivars can be shown to differ isozymically or morphologically, the need for time-consuming and expensive DNA fingerprinting studies is eliminated. Only where cultivars cannot be discriminated with isozymes or morphology are more detailed genetic tests required to verify the hypothesis of clonal identity.

The three *Salvia mellifera* cultivars are prostrate forms from Monterey County, however two appear to represent identical clones while 'Little Sur' is genetically distinct. Likewise, *Fremontodendron* 'Ken Taylor' (Tree of Life), 'Ken Taylor' (UC Santa Cruz), 'West Hills Hybrid', and 'El Dorado Gold' all are supposed to represent F_1 progeny of *Fremontodendron californicum* ssp. *decumbens* × *Fremontodendron* 'California Glory'. However, 'Ken Taylor' (Tree of Life) and 'El Dorado Gold' are morphologically identical, whereas the other two siblings are quite distinctive. Thus, the genetic data confirming the clonal nature of the former was anticipated.

What was more surprising was the fact that the *Heuchera* and both *Ceanothus* cultivar pairs are clearly different genetically. Both *Heuchera* 'Susana' and *H.* 'Santa Ana Cardinal' are believed to be clonally propagated F_1 hybrids of *H. maxima* × *H. sanguinea.* Given their distinctive RAPD profiles, however, it is likely that the cultivars are derived from two independent matings between these two species. Both pairs of morphologically similar, clonally propagated, hybrid *Ceanothus* cultivars also appear to represent independent crosses between their hypothesized parental species (*Ceanothus impressus* × *C. papillosus* Torrey & A. Gray var. *roweanus* McMinn ⇒ 'Dark Star' and 'Julia Phelps' and *C. thyrsiflorus* × *C. velutinus* Hook ⇒ 'Skylark' and Victoria').

Based on these results, separate cultivar names for *Salvia mellifera* 'Prostrata' and 'Tera Seca' and *Fremontodendron* 'Ken Taylor' (Tree of Life) and 'El Dorado Gold' should not be maintained. Thus, we place *Salvia mellifera* 'Prostrata' in synonymy with *S. mellifera* 'Tera

Seca' because the latter represents the first nomenclaturally valid name for this cultivar. Likewise, *Fremontodendron* 'Ken Taylor' (Tree of Life) has been incorrectly identified and should be treated as synonymous with *Fremontodendron* 'El Dorado Gold'.

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